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The roles of vitamin B12, 25(OH)D, and folate in primary nocturnal enuresis: A single center experience

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Abstract

Aim: In this study, we investigated the connections between children born late preterm (LPT) and their susceptibility to nocturnal enuresis, examining their vitamin B12, folate, iron, and 25(OH)D levels.

Material and Methods: From April 2018 to December 2020, the research group comprised 146 children with PNE who appeared at state hospital urology clinics, whereas the control group included 102 healthy children who presented at the pediatric clinics. Primary nocturnal enuresis was characterized as having more than four wet nights per week in children aged 5 to 13 years. Both groups' hospital records were compared in terms of age, height, weight, vitamin B12, folate, ferritin, and iron levels.

Results: There was no difference found in demographic characteristics between our study and control groups. In agreement with the literature, the mean vitamin B12, 25(OH)D, and folate levels were significantly lower in the enuresis group than in the control group.

Discussion: The delayed CNS development or the onset of sleep problems may be caused by low levels of vitamin B12, folate, and 25(OH)D in PNE patients. To find out how PNE and folate levels are related, a more extensive series is required.

Keywords

Vitamin B12, 25(OH)D, Folate, Iron, Primary Nocturnal Enuresis

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Introduction

Nocturnal enuresis (NE) is a term used to describe involuntary urination during sleep in a child who is five years of age or older and does not have any urogenital malformations or congenital or acquired central nervous system problems [1,2]. Enuresis, in conjunction with daytime voiding situations, is viewed as significant if more bedwetting episodes occur once per month and a minimum frequency of three times per 3 months [3]. It is undeniable that PNE has both psychological and economic effects on children and families. Nevertheless, despite its predominance, its pathogenesis remains unclear.

The pathogenesis of PNE has been linked to a delay in the maturation of the central nervous system. In previous studies, anomalies in arginine vasopressin secretion, bladder dysfunction, and sleep disorders have also been implicated in the development of PNE [4,5]. However, a role for vitamin and mineral nutrition has also been proposed.

Similarly, rodent models have also shown that iron deficiency during pregnancy and lactation alters myelination, neurotransmitters, neurometabolic, and gene/protein profiles before and after iron replenishment at weaning. Infants with iron deficiency anemia (IDA) had lower social-emotional, neurophysiological, cognitive, and motor development test results compared to the control group [6]. Iron, vitamin B12, and folate are vitamins essential for the development of the nervous system. These findings highlight the role of vitamin nutrition in PNE.

Recent evidence suggests that vitamin 25 (OH) D deficiency in children may be a risk factor for nocturnal enuresis [7]. Vitamin D regulates calcium excretion in the proximal tubule by affecting calcium-sensing receptor genes, thus indirectly affecting fluid retention [8]. Some investigators have demonstrated the relationship between nocturia and obstructive sleep apnea [9], which is a consequence of low serum levels of vitamin 25(OH) D in children [10].

No studies in the English literature have evaluated concomitant blood vitamin B12 and 25-hydroxy vitamin D levels in children with PNE, to our best knowledge. Thus, the purpose of this study was to compare vitamin 25(OH)D, vitamin B12, folate, and iron levels in pediatric patients with PNE with those in otherwise healthy pediatric participants.

Material and Methods

Our study used a retrospective cohort approach. After obtaining the ethics committee's approval for the study, data were gathered from the electric clinical records of the participating government hospital. We searched the health administrative data using the International Classification of Diseases-10 (ICD-10) codes. The integrity of each diagnosis was changed twofold and checked freely by 2 authors who analyzed the medical records and discharge reports. All the findings of the selected patients were deemed appropriate.

Study population

Patients with monosymptomatic NE, aged 5 to 13, who claimed over four wet nights per week and visited the government hospital between April 2018 and December 2020, were included in our study. A total of 248 children were included in this study, out of which 146 children aged between 5 to 13 years and diagnosed with PNE were included. The sample included 52 girls and 94 boys. Additionally, 102 children who did not have any disease and were selected for routine health checks among healthy children in outpatient clinics were also included in the study. These children, comprising 47 girls and 55 boys who did not have enuretic problems, were compared with the other groups to investigate their epidemiological information and clinical outcomes. The control group was randomly chosen according to the strategy of block randomization to create sample groups of equivalent example sizes from the hospital information system. We excluded those with a history of congenital kidney abnormality as well as urinary tract, neurologic abnormalities, any chronic disease, previous urinary tract infection, or a history of glomerular disease.

Examinations

Senior urologists and pediatricians treated all patients. While detailed physical examinations of the children were made and their age, gender, height, and weight values were noted, data such as bowel habits, family history of enuresis, frequency of daytime urine, and several wet nights were obtained from the interviews obtained from the mothers of the children. Blood tests were taken toward the beginning of the day after around 8 hours of fasting. Serum vitamin D levels were evaluated with the 25-hydroxy vitamin D (Monobind, USA) pack. All participants' serum vitamin B12, vitamin D, folate, ferritin, and iron levels were assessed. Results are displayed using the mean ± standard deviation (SD) and ranges.

Statistic

The statistical program SPSS, version 25.0, was used to analyze the data that had been collected (SPSS Inc., Chicago IL, USA). Paired samples t-test and Mann-Whitney *U* test were utilized for statistical evaluation. For statistical significance, a difference had to be P 0.05.

Ethical Approval

This study was approved by the Ethics Committee of Fırat University Faculty of Medicine (Date: 2020-11-12, No: 97132852/050.01.04/)

Results

Table 1 shows the overall characteristics of the groups. The PNE group had an average age of 9.4 ± 2.79 years, while the control group had an average age of 8.6 ± 2.69 years. Age, gender, bowel habits, body mass index, number of wet nights per week, and urine osmolality did not differ substantially across research groups. There were no variations in biochemically mean hemoglobin, hematocrit, or mean corpuscular volume (MCV) values between the PNE and control groups (P > 0.05; Table 1). Enuresis was present in 83.56% of the parents in the enuresis group, which was considerably higher than in the control group. (P 0.001; Table 1)

The median level of serum 25(OH)D was 26.2 ng/ml. Regardless of seasonality, we discovered a significant difference between the PNE and control groups when vitamin 25(OH)D levels were compared between the two groups (p=0.007; Table 1). Mean 25 (OH) D levels were 24.24 ± 7.95 for PNE and 32.11 ± 11.24 for the control group.

Likewise, mean B12 and folate levels in the enuresis group were considerably lower than in the control group (p=0.001 and p=0.029, respectively) (Table 2). Table 2 provides a statistical comparison of serum ferritin and iron levels between the groups.

Discussion

This investigation led us to the realization that the blood levels of 25(OH)D, B12, and folic acid in children with NE were considerably lower than those in the control group. However, to the best of our knowledge, there is no published research from reliable English sources on the topic of vitamin D, B12, folate, and iron intake among patients with PNE. As a result, this study is the first of its kind.

The International Children's Continence Society (ICCS) defines primary nocturnal enuresis as a symptom of intermittent incontinence during sleep [2]. Despite studies on this subject, the etiology of enuresis has not been elucidated. This is probably because of its multifactorial etiology. Psychogenic and behavioral components, delayed central nervous system maturation, sleep apnea, environmental effects, and deep sleep are among the most accused pathologies [9-10].

Early detection and treatment of VitB12 deficiency are critical in children and pregnant women to prevent severe anemia and permanent neurological deficiencies. Clinical research has provided strong evidence that enuresis treats developmental delay or maturation lag in central nervous system development [11]. The functional maturation theory states that delayed central nervous system control over the bladder at night is the leading factor in the pathophysiology of nocturnal enuresis [12,13].

Table 1. Comparison of the primary nocturnal enuresis (PNE) group and the control group in terms of basic characteristic

Variable		Enuresis	Control	P value
Children	Boy, n(%)	94 (64.4)	55 (53.9)	0.069
	Girl, n(%)	52 (35.6)	47 (46.1)	
Race	Turkish n(%)	133 (91.1)	96 (94.1)	<0.001
	Others n(%)	13 (8.9)	6 (5.9)	
Age, year, (mean±std)		9.4 ± 2.79	8.6 ± 2.69	0.064
Weight, kg, (mean±std)		31.2 ± 9.58	30.79 ± 9.81	0.286
Height, cm, (mean±std)		134.92 ± 12.98	133.57 ± 11.93	0.132
Parental history of enuresis (%)		122 (83.56)	11 (10.78)	<0.001
Hb (gr/dl)		13.12 ± 0.89	12.32 ± 1.2	0.362
Hematocrit (%)		39.26 ± 2.12	38.37 ± 2.76	0.096
MCV (fL)		80.34 ± 3.63	79.35 ± 5.38	0.169

Table 2. The comparison of 25-OH D, Vitamin B12, Folate, Fe, Fe binding protein, and Ferritin levels of primary nocturnal enuresis and the control groups.

Variable	Groups	Mean	Std. Deviation	P value
25-OH D (mg/L)	Enuresis	24.24	7.95	0.007
	Control	32.11	11.24	
B12 (pg/ml)	Enuresis	334.85	128,66	0,001
	Control	409.94	153,18	
Folate (ng/ml)	Enuresis	8,96	2,84	0,029
	Control	13,93	2,37	
Iron (ng/ml)	Enuresis	85,95	46,91	0,059
	Control	77,71	28,17	
Fe_Binding (ng/ml)	Enuresis	286,57	53,74	0,263
	Control	296,71	59,5	
Ferritin (ng/ml)	Enuresis	37,83	20,77	0,885
	Control	38,22	21,98	

While vitamin B12 plays an important role in many neurological events, especially in the development of the central nervous system, its deficiency can cause a wide range of neurological symptoms, from cognitive and behavioral changes to neural tube defects [11]. Similar to vitamin B12, folate is an essential vitamin for the proper development of the central nervous system, especially during early pregnancy, as folate deficiency can cause congenital neural tube defects. Folate deficiency, such as vitamin B12 deficiency, can cause neural pathology, especially cognitive dysfunction and dementia. Evidence for this is a demonstration of behavioral abnormalities in folate-deficient mice [14]. The uncertain etiopathogenesis of enuretic children has led us to measure vitamin B12 and folate levels, as they are effective in the maturation of neurogenic functions. Altunoluk et al. observed that vitamin B12 and Folate levels in the enuresis group were significantly lower than those in the control group in their study evaluating the effectiveness of B12 and folate in the etiology of NE in children [15]. Similar results have been reported by Albayrak et al. [16].

Li et al. discovered in their experimental investigation that mice lacking the vitamin D receptor had significantly higher 24-hour urine volume than the control group [17]. Similarly, a negative correlation has been demonstrated between serum vitamin D levels and nocturnal enuresis [7]. According to Kong et al., a normal quantity of vitamin D decreases renin gene transcription through the cAMP pathway. Polyuria occurs because of vitamin D receptor deficiency through an increase in renin [18]. Rahmani et al. asserted, however, that giving infants nocturnal enuresis vitamin D supplements considerably decreased the number of attacks [19]. Parallel to research in the literature, this study found that vitamin D levels in enuretic children were considerably lower in the control group.

Previous studies have reported that iron deficiency, such as vitamin B12 and folate deficiency, negatively affects neural maturation. In studies on this subject, impaired autonomic balance in favor of parasympathetic activity has been shown in children with iron-deficiency anemia [20]. In animal studies, various disorders have been observed during the development of the central nervous system in patients with gestational iron deficiency [21]. Given these findings, even though it is anticipated that iron deficiency anemia may contribute to the etiology of nocturnal enuresis, similar to the findings of Albayrak et al., blood iron and ferritin levels in children with enuresis were shown to be greater than those in the control group [16].

Another parameter investigated in the etiology of NE is family history. If one of the parents had a positive history, the incidence of NE in children was 44%, while this rate could reach up to 77% in children with a positive history in both parents [22]. Despite these available data, many researchers still believe that PNE is caused by the disruption of maturation of the central nervous

system connections involved in bladder control [13].

Overall, while looking at children with NE in clinical practice, the coexistence of vitamin B12 and/or vitamin 25(OH)D deficiency should be followed if the patient has a high frequency of bedwetting.

Strengths and limitations

Our study offers certain key advantages. This is the first investigation, as far as we are aware, on the relationship between concurrent serum 25(OH)D and B12 levels in children with PNE. The study's weaknesses are the limited sample size, retrospective design, and lack of consideration for climatization and seasonality. We utilized retrospective data from medical clinic records of our study group, social status, time spent under sunlight, parathyroid (PTH) levels, dressing habits, and children's daily 25-hydroxy vitamin D intake. We have not assessed the long-term results or investigated the daytime urine volume. The inability to evaluate the effect of supplementation in children with low levels of these vitamins on the treatment of PNE can be considered another limiting factor.

Conclusions

Vitamin D, B12, and folate levels were considerably lower in children with PNE than in the control group in our research. We believe that delayed CNS development may be related to the low levels of 25-hydroxyvitamin D, B12, and folate in children with PNE. Large series and controlled trials in which the indicated vitamins will be employed in the treatment of patients with PNE with low vitamin D, B12, and folate levels will shed further light on this problem.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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